# Use of Nanoshells for Combined Two-Photon Imaging and Therapy of Breast Cancer

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## Introduction

Development of superior diagnostic and therapeutic tools for cancer is crucial. Nanoshells, spherical nanoparticles consisting of a dielectric core and a thin gold shell, can be synthesized to absorb light in the near-infrared, a region where light penetrates deeply into tissue, rendering them useful for near-infrared photothermal cancer therapy.[1,2] In this work we studied two types of nanoshells, one with a gold sulfide core (total diameter ~50 nm) and one with a silica core (total diameter ~150 nm). In the current work, we examined the use of nanoshells as a combined imaging agent and therapeutic. Upon excitation with a pulsed laser, silica nanoshells exhibit two-photon induced photoluminescence which may be used to image cancer cells.[3] In addition, by increasing the power output of the laser, cancer cells can be thermally ablated as nanoshells convert the light energy into heat.

## Materials and Methods

*Silica Nanoshell Synthesis*

Nanoshells with silica cores (120 nm diameter) and ultrathin gold shells (14 nm) were manufactured as previously described.[4] After functionalizing the cores with amine groups, colloidal gold particles (~3 nm) were adsorbed to the surface. The shell was completed by reduction of additional gold, producing particles with peak absorption at 800 nm.

*Gold-Gold Sulfide Nanoshell Synthesis*

Nanoshells consisting of a gold sulfide ($Au_2S$) core and gold shell were made following literature techniques.[5] Solutions of 2 mM $HAuCl_4$ (Alfa Aesar) and 1 mM $Na_2S$ (Aldrich) were mixed at a volumetric ratio of 1:2 ($Na_2S$:$HAuCl_4$), resulting in nanoshells resonant at 800 nm.

*Antibody Conjugation*

Anti-HER2 antibody (NeoMarkers) was conjugated to nanoshells using a poly(ethylene glycol) (PEG) linker purchased from Creative PEGWorks, with an N-hydroxysuccinimide terminus for antibody coupling and a disulfide terminus for attachment to gold. To prevent non-specific interactions, PEG-SH was added to the surface of the nanoshells after antibody addition. Control nanoshells were coated with only PEG-SH.

*In Vitro Study*

SK-BR-3 breast carcinoma cells, which over-express the HER2 receptor, were incubated with either anti-HER2 or PEG-SH coated nanoshells in suspension for 30 minutes. Unbound particles were removed by centrifugation and cells were cultured on coverglass overnight to allow time for adhesion. Samples were then imaged and treated with a Zeiss LSM 510 Meta multiphoton system operated with a Coherent Chameleon femtosecond Ti:sapphire laser. For imaging the laser output was 60 µW at 800 nm. To perform ablating therapy the power was increased to 240 µW. Samples were exposed to the laser for 15 seconds. Following an incubation period of one hour, cell viability was assessed with calcein AM staining.

## Results

SK-BR-3 cells could be visualized by two-photon microscopy only in the presence of anti-HER2 nanoshells. Images for gold-gold sulfide nanoshells are shown in Figure 1. Results for silica nanoshells were similar.

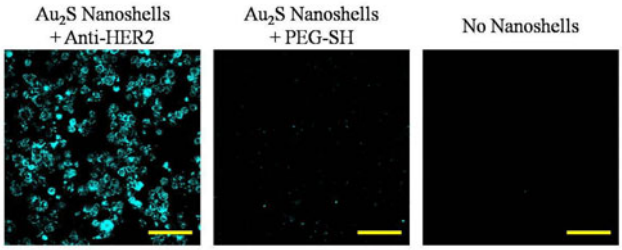


Figure 1. Two-photon images of SK-BR-3 cells. Anti-HER2 coated nanoshells provide the best contrast. Scale bar = 100 µm.

Calcein AM viability staining verified that the laser power used for imaging (60 µW) was not harmful to cells. Upon increasing the power output to 240 µW, cell death was induced only when targeted nanoshells and laser exposure were combined (Figure 2).

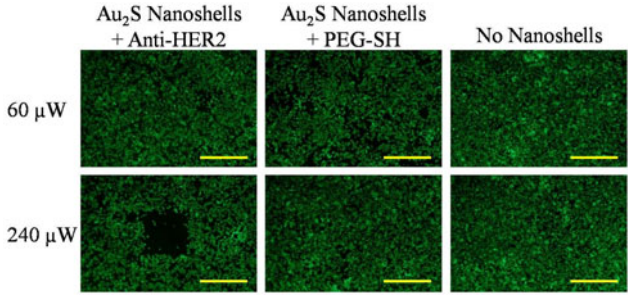


Figure 2. Cell death was observed only when targeted nanoshells were combined with higher laser powers, as seen by loss of fluorescence in the bottom left image. Scale bar = 500 µm.

## Conclusions

Nanoshells can be combined with two-photon microscopy to provide both imaging and therapy of cancer. Images of cancerous cells can be obtained at low laser powers, and then by increasing the power output cancerous cells can be destroyed by photothermal ablation. In the future, this technique could be used to treat tumors immediately upon detection.

## References

1. Hirsch LR. PNAS. 2003; 100: 13549-13554.
2. Weissleder R. Nat Biotechnol. 2001; 19: 316-317.
3. Park J. Opt Express. 2008; 16: 1590-1599.
4. Oldenburg SJ. Chem Phys Lett. 1998; 288: 243-247.
5. Averitt RD. J Opt Soc Am B. 1999; 16: 1824-1832.